Performing the EP stray-light test with potassium chloride on UV-visible spectrophotometers

Technical Note

Introduction

Measured stray light has two components:

- light coming from the light source of the instrument that lies outside of the bandwidth of the selected wavelength, λ_n
- ambient light that reaches the detector either directly or by simple reflections, see figure 1

For instruments with reversed optics such as the Agilent 8453 UV-visible spectrophotometer[1] ambient light can not reach the detector, see figure 2. The equation used to calculate transmittance and thereby absorbance is:

$$T = (I + I_s)/(I_0 + I_s)$$

Where T is transmittance, I_0 is intensity of incident light, I is intensity of transmitted light and I_s is intensity of stray light. Stray light has an increasing influence on spectroscopic measurements at low levels of intensity, that is, high absorbances.

The result is that stray light causes a negative bias in instrument response and eventually becomes the limiting factor for absorbance and therefore concentration that can be measured. The effect of various levels of stray light on measured absorbance compared with actual absorbance is shown in Figure 3.

This Technical Note examines the influence of sample composition, sample temperature, bandwidth and wavelength accuracy on the result of the stray-light test using potassium chloride as described in the European Pharmacopoeia (EP).[2]

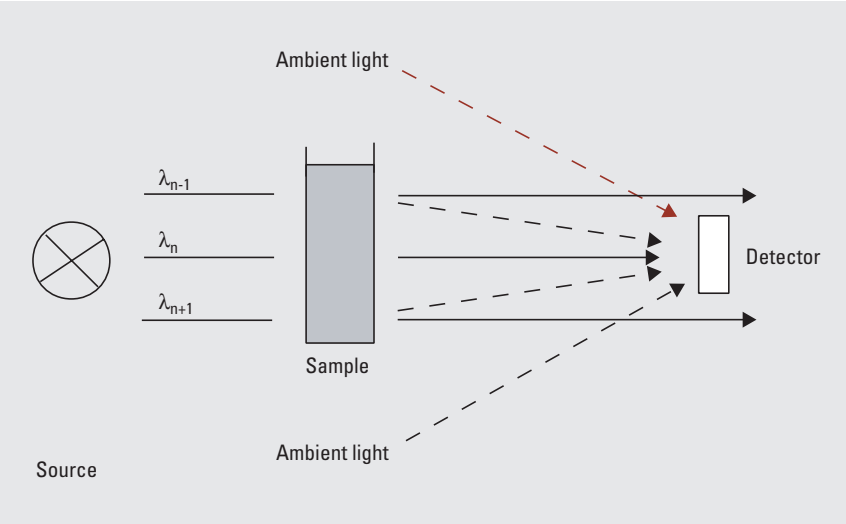

Figure 1
Terminology used when describing stray light

Measurement of stray light

To measure stray light, a filter is required that absorbs all light of the wavelength at which the measurement is to be made and transmits higher and lower wavelengths. Figure 4 shows this ideal stray light filter. At the measured wavelength (200 nm) the transmission is 0% whereas at all other wavelengths it is 100%. Such filters do not exist in practice, so cut-off filters are used which transmit all light above a certain wavelength and block all light at lower wavelengths.

Salt solutions, for example, potassium chloride (12 g/l), sodium iodide (10 g/l) and sodium nitrite (50 g/l) in water, can be used as standard stray-light filters at 200, 220 and 340 nm respectively (see figure 5).

The user should keep in mind that all 3 filters are only approaches to the ideal test filter. The slope of the absorption edge shows no infinite value like the ideal filter does. In addition the used filters block all light from wavelengths shorter than the measured one. The contribution of stray light that might result from these wavelengths is thus eliminated. This leads to smaller stray light values than would be expected from the ideal filter. The user should be aware of this systematic deviation as a consequence of using non-ideal stray light filters.

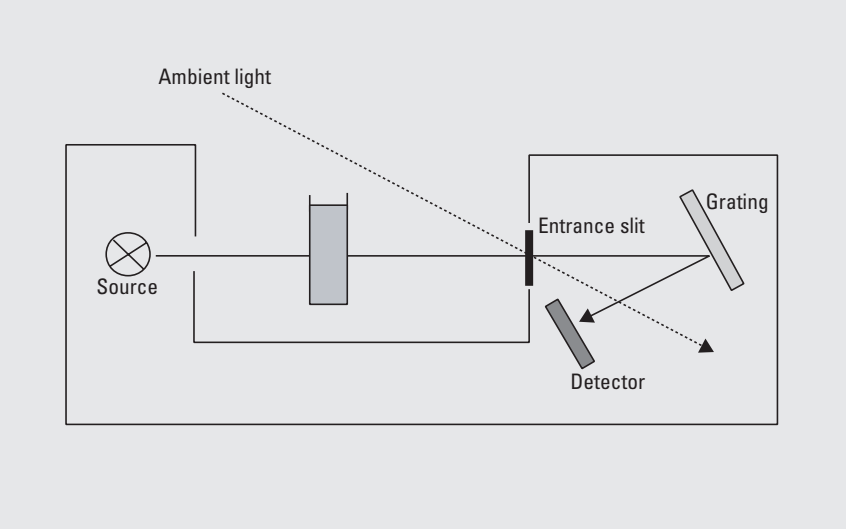

Figure 2
Simplified schematic of a diode-array spectrophotometer

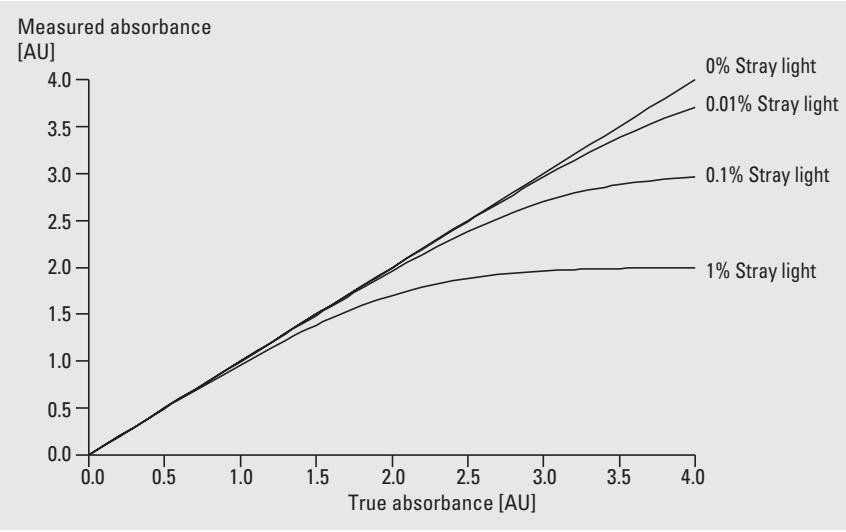

Figure 3
The effect of stray light on measured sample absorbance

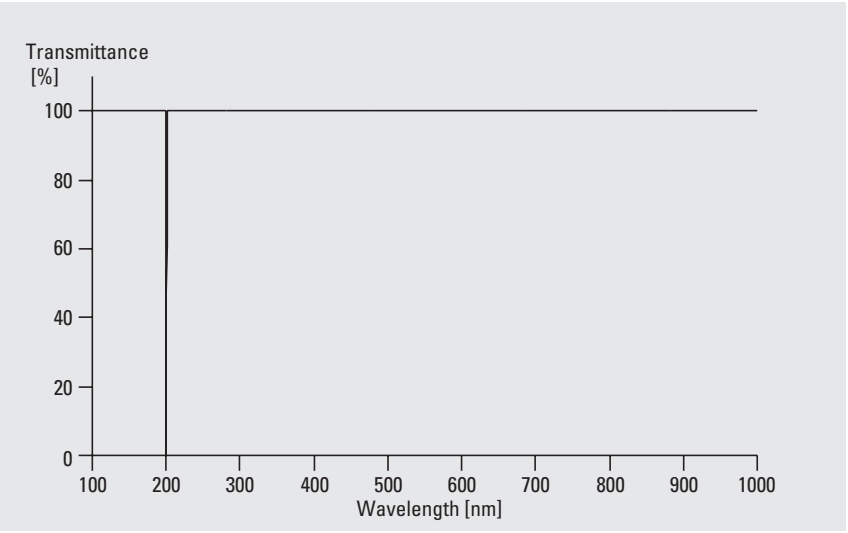

Figure 4
The ideal spectrum of a stray-light filter at 200 nm

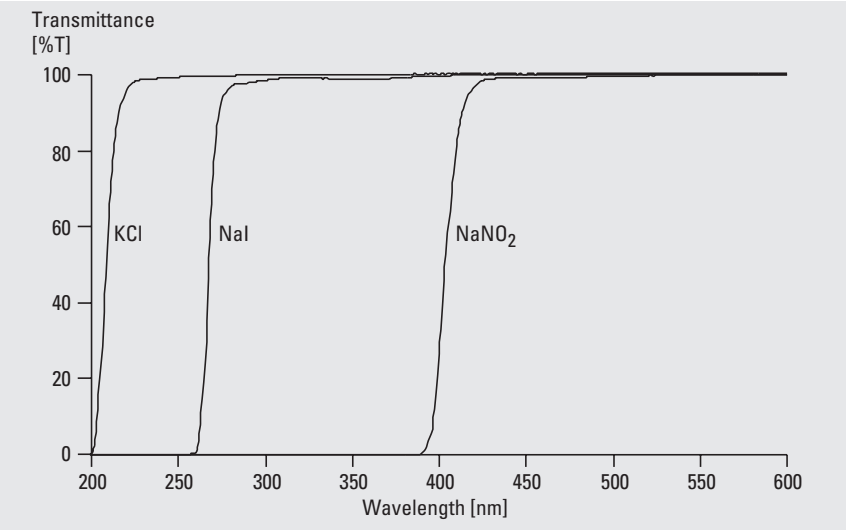

Figure 5
The spectra of potassium chloride (12 g/l), sodium iodide (10 g/l) and sodium nitrite (50 g/l) in water

The EP stray-light test

Stray-light measurement with the Agilent 8453 UV-visible spectrophotometer comprises the three test described above. The potassium chloride test is specified in the EP[2]:

"…Stray light may be detected at a given wavelength with suitable filters or solutions: for example the absorbance of a 12 g/l solution of potassium chloride in a 1 cm cell should be greater than two at 200 nm when compared with water as the compensation liquid."

An absorbance of greater than two means a transmittance of less than one percent. For a number of reasons the potassium chloride test is the most critical of the three stray-light tests. As shown in figure 5, this is the only test in which the measured wavelength is situated very close to the cut-off slope. As a consequence the test is extremely sensitive to the wavelength accuracy of the spectrophotometer. Even small deviations will result in test failure. For this reason a wavelength recalibration should be performed before each stray-light test.

Further, the measurement time is also relevant. When measuring the transmittance with the test solution in the light path the intensity at the detector is very low. The signal-to-noise ratio can be improved by increasing the integration time of the spectrophotometer. All three stray-light tests with the Agilent 8453 spectophotometer were performed using integration times of 5 s.

Another important issue is the quality of the selected potassium chloride. The concentration of bromide plays an important role on the position of the absorption edge. The EP allows a maximum of 0.15 % potassium bromide (0.1 % bromide)[2]. Figure 6 shows spectra of potassium chloride solutions with different bromide percentages measured on the Agilent 8453 spectrophotometer. From left to right the bromide concentration is increasing. Spectra 1 to 3 shows the transmittance of potassium chloride samples with bromide contents of <0.005%, 0.05% and 0.10%, respectively.

Increased bromide concentrations lead to lower percentage transmittance values at 200 nm (see table1), because the wavelength at 50% transmittance is gradually shifted from 204.9 to 210.7 nm. Spectrum 4 shows a non-EP conform sample with bromide content above the EP limit of 0.1% and thus resulting in lower transmittance value at 200 nm. Table 1 summarizes the stray light data of the different samples.

It should be emphasized that problems can arise when using highly pure potassium chloride samples. At a very low bromide concentrations the transmittance value at 200 nm may exceed the EP limit of 1%. It would make no sense to use ultra-pure potassium chloride samples for stray light analysis, although the quality of the chemicals are according to the specifications of the EP.

Bromide [%]	T <200nm>[%]	Wavelength <T=50%> [nm]
<0.005	>0.64139	204.9
0.05	0.25893	208.3
0.10	0.18658	210.7
>0.15	<0.15534	217.6

Table 1
Measured stray light on the Agilent 8453 spectrophotometer as a function of bromide concentration (blank on water)

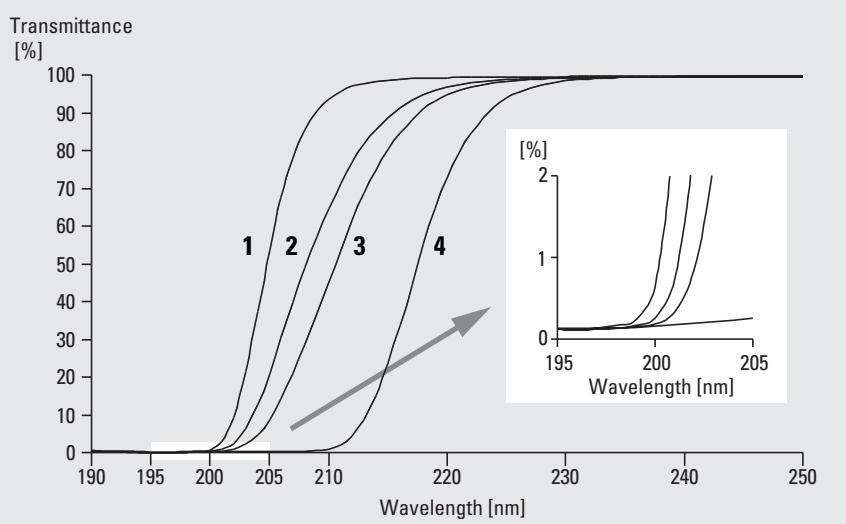

Figure 6
Potassium chloride samples measured on the Agilent 8453 spectrophotometer: Influence of increasing bromide concentration on the shape of the absorption edge
1: < 0.005%
2: 0.05%
3: 0.10%
4: >0.15%

Slit width <nm>	%T <200nm>	Wavelength <T=50%> [nm]
0.5	0.0111	210.8
1.0	0.0169	210.7
1.5	0.0286	210.7

Table 2
Stray light data of a conventional scanning spectrophotometer with variable bandwidth.
Sample: potassium chloride (bromide: 0.10%),
Blank: water

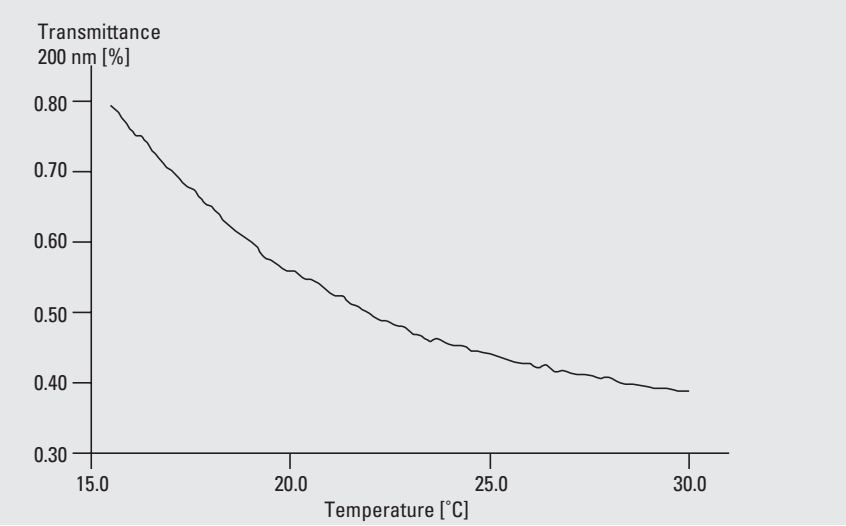

Figure 7
The effect of temperature variation on the transmission of potassium chloride solution at 200 nm

Another factor that has a lasting effect on the measured transmittance at 200 nm is the spectral bandwidth (SBW) of the spectrophotometer. Decreasing the SBW leads to decreasing readings when the natural bandwidth (NBW) of the absorbing substance and SBW have the same order of magnitude. However, the wavelength at 50% transmittance is virtually independent of the selected bandwidth. These dependences are shown in table 2 for the KCl solution with 0.1% bromide. The data were aquired with a high performance conventional scanning spectrophotometer that possesses a better stray-light specification than the Agilent 8453 spectrophotometer.

The SBW of the Agilent 8453 spectrophotometer is comparable to a conventional scanning instrument with a spectral bandwidth of about 1.5 nm. The bandwidth is fixed and cannot be changed by the operator.

The temperature also strongly affects results of stray-light measurements, see Figure 7. With increasing temperature the absorption band is broadend, leading to smaller transmittance values at 200 nm. The EP recommends a temperature of 20°C ±1°C for the potassium chloride stray-light test.

ASTM stray light tests[3]

The sodium nitrite and iodide tests both have cut-off wavelenghts that are far away (50 and 60 nm) from the measured wavelenths (see figure 5). For this reason the two tests are less sensitive to wavelength accuracy of the spectrophotometer. On the other hand both stray light tests vary more from the ideal test filter than the EP test.

Summary

Many factors influence the collected stray-light data. When using liquid filters such as potassium chloride users should be aware that they are applying non-ideal stray-light filters. For the EP stray-light test, the obtained results depend not only on several external factors like sample temperature and wavelength accuracy of the spectrophotometer, but also to a high degree on the bromide content of the potasssium chloride used.

The EP allows a maximum of 0.1% bromide. Independent from the spectral bandwidth of the spectrophotometer, a bromide concentration of maximum 0.1% corresponds to a wavelength at 50% transmittance of less than 211 nm. If the potassium chloride solution used shows a wavelength at 50% transmittance that is more than 211 nm, it does not conform to the requirements of the EP.

The Agilent OQ/PV standards kit (order number 5063-6503) offers an easy-to-use, cost-effective and fully-EP-compliant solution for stray-light determination of UV-visible spectrophotometers. All three solutions (KCl, NaI and $NaNO_2$) are part of this kit and are provided in sealed ampules for use with standard 10 mm quartz cuvettes.

References

1. HP 8453 Spectrophotometer and Open Sample Area, *Agilent Technologies Technical Note*, **1999**, publication number

2. European Pharmacopoeia, third edition, **1997**, pages 29 and 1361

3. ASTM E 387-84, Standart Test Method for Estimating Stray Radiant Power Ratio of Spectrophotometers by the Opaque Filter Method

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Printed in Germany 11/2000
Publication Number 5988-0945EN

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